THE EXPERIMENTAL MODIFICATION OF GERM-PLASM

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The doctrine of an inviolable germ-plasm has formed the foundation of many imposing edifices in biological thought, and facilitated many advances in genetics and heredity during the last two decades. The authors who have rigidly adhered to the principles of the hypothesis and reasoned from its tenets have exposed many fallacies which have been offered in explanation of problems in evolution.

This prevalence of theoretical considerations over mistaken experiences has laid the foundation for an unreasoning devotion to the idea of an independent germ-plasm, carrying agents which may not be seen, measured, or tested in any practicable manner, and which might consequently be termed "idealoplasm" with attributes approaching the supra-physical.

The desperate straits of those who voluntarily consign themselves to the bondage of such a conception is well exemplified by the group of writers who subscribe to the conclusion that all evolutionary movement is due simply to recombination and rearrangement of qualities or factors already present in the protoplasm. An additional illustration of the futile extremes to which this view may be pushed is to be found in the recent utterances of Bateson, who has arrived at the conclusion that evolution is mainly and essentially loss of inhibitors, and release of activities previously latent or suppressed, an hypothesis which predicates premutation.

If it be allowed that the non-appearance of a character is a direct loss of its determiner and that the appearance of a new feature is the loss of a retarder or inhibitor which held it in abeyance, then the answer to the question as to the method by which organisms have arrived at their present condition is obvious, but of a simplicity that is metaphysical instead of actual and hence of little value, even tentatively, as a framework on which new concepts in biological science may be formulated. The group of problems with which we are endeavoring to make headway are in the domain of physiology and their solution may be reached only by experimentation, the results of which are to be interpreted in terms of physico-chemical activities and their correlated functional manifestations in the living organism.

That phylogenetic advance in the main lines of descent in the plant kingdom at least reflects, or harmonizes with, the expectancies of somatic experience is tacitly admitted on all hands, but that the direct response of a shoot to the environment, or conversely stated, that the impression on the soma made by environic agencies is communicated to successive generations in a constant manner has not been demonstrated, although it seems fairly established that certain experiences of individual plants are reflected directly or indirectly to the next generation, and in lesser degree to the next or second generation. How are lasting or permanent changes brought about?

Functional adequacy and architectural suitability present themselves on every hand, yet about all of our reliable evidence is against anything like a direct or functional adaptation becoming hereditary or continuously transmissible.

Two methods of experimental attack on the problem are available. Species showing measurable features and of simple genetic constitution may be taken from their habitual or known environment to other localities in which the climatic and soil characters may be calibrated and the response of the organism, somatically and hereditarily, determined. Hundreds of thousands of introductions and acclimatization operations have been carried out in agriculture, horticulture, and especially in botanic gardens during the last century, yet neither the genetic constitution nor the response of the organism has been followed by trained observers who compared the plants in their different habitats. The exposure of the organism to any climatic complex, of course, might affect the germ-plasm directly, and any departure detected in such experimentation must be evaluated by controlled cultures under laboratory conditions in which both the nature of the reaction and the identity of the inciting agent may be found. The most notable series of experiments of this character which have as yet been carried out are those of Tower with the potato beetles.

Over two hundred species of seed-plants selected for their suitability and promise of response have been taken into the series of cultures of the Department of Botanical Research on mountain top, desert, and at the sea-shore, less than eighty of which have survived and about a score continue in all three locations. The most notable feature in the behavior of these plants put under stress in unaccustomed habitats consists in divergences in sexual reproduction and seed-formation. Conjointly with this decrease of the sexual reproduction, vegetative propagation assumes a greater importance. Shoots are variously affected. The measurement of these departures and their fate when the *n* th generation is returned to the original habitat, or to a place in which the habital tension is changed, will be necessary to determine whether or not permanent impress on the species has been made.

The second method would include all forms of experimentation in which inciting agents would be applied directly to the reproductive bodies, in which case any deviation from the usual or typical would be more clearly attributable to changes in the germ-plasm.

It is pertinent to call attention to the necessity for new viewpoints and new standards in the evaluation of any results which may be obtained in such manner. We are not likely to go far or progress easily into the region of the unknown if we attempt to interpret these effects too directly, with the idea that determiners, inhibitors, genes, etc., are ultimate or even penultimate units. In brief, the time has come for testing the performances of lineal series of organisms by methods in which attention will be centered upon the physico-chemical complex and an open eye will be kept for cleavage lines which may cut across directly or obliquely the limits of all of the arbitrary concepts of alternate inheritance. The house of the living thing is inclusive of walls, doors, roofs, windows, floors, ceilings, rafters, and plumbing, but the materials used may be bricks, stones, metals, sand, lime, boards, glass, and paint. Our present needs lead us to experiments with these components rather than to trials of the possible combinations and inhibitions, possibilities and impossibilities of sets of builders' blocks, no matter how complete or full these may be.

Living material is a colloidal complex with its enmeshed reactions highly fluctuant, its combinations unstable and its types of energy transformation multifold. It is concrete, however, and amenable to experimentation of many kinds. Its physical qualities and form undergo changes of phase which have some correspondence with the mechanism of morphogeny, reproduction, and heredity. Thus, for instance, in the higher plants the germinal protoplasm in the earlier stages of the individual is in the form of meristematic tracts made up of highly distended plasts in which absorption of water, hydratation, auxetic enlargement, and division of the separate elements is very marked and rapid. Elements at the peripheries of these masses are separated which undergo differentiation and pass into the permanent tissues of the individual. These separating cells may be modified to an enormous extent by external agencies; thus conditions of aridity acting upon an individual may cause the tissues formed from its embryonic tracts to make such structures as to give the organs which they make up a xerophytic aspect.

This final xerophytic or other character of the soma, however, is in the permanent tissue, and the modifications which have resulted in its specialization ensued after the cells were pushed away from the meristem, and there seems to be no reflection of the final fixed qualities back to the embryonic tract, although there are many promising possibilities to be considered. Of these none are more interesting than the regenerative processes by which highly specialized cells reassume embryonic activity and reproduce members or individuals vegetatively. Actual tests of the transmission and permanence of the specializations under these conditions have not yet been made with that exactitude which would allow any serious conclusion to be formulated. At certain stages of the ontogeny, generally much later in the plant than in the animal, and this is a matter which may be determined by the environic agencies, the germ-plasm or meristem tract undergoes such change of phase that instead of all of its separating elements passing into somatic cells a few become reproductive masses from which sexually specialized elements may be differentiated, and in which the number of chromosomes, the metabolic balance, degree of hydratation, auxetic energy and mechanism of division suggest physico-chemical conditions widely different from those of somatic elements; furthermore, the reproductive elements are highly individualized. The meristem in its myriad cells may at any moment present all of the phases of growth and differentiation. The egg nucleus or the fertilized egg, a single element of the plasma, may include the fate of the individual and its unending line of progress, and it may be affected in its entirety by agencies impinging upon it. The reaction of such specialized cells to external agencies would of course be different from those of the meristem tracts, which are made up of plasmatic units of the most generalized form.

The experiments of Tower with the *Leptinotarsae,* which have been carried on under widely diverse conditions in southern tropical Mexico, in the arid semi-tropical climate of the Desert Laboratory, and under controlled conditions at the University of Chicago, furnish a great series of cultures of these beetles in which it is possible to demonstrate logically by exclusion and analysis that certain climatic features, notably moisture, may affect the germ-plasm, or the entire organism when the germ-plasm is in a certain stage, in such manner as to induce disturbances in hereditary lines. These experiments show the vulnerability of the germ-plasm.

That the germ-plasm is directly responsive to the action of foreign substances which are introduced into the embryo-sac was demonstrated when (early in 1905) I was so fortunate as to hit upon an experimental method of treatment of the ovaries of seed-plants which resulted in the formation of embryos developing into individuals not entirely identical with the parental types. The essential feature of the discovery consisted in the successful introduction of various substances into the neighborhood of the embryo-sacs at the time that fertilization was imminent, and when the first trials were made I had two main purposes in mind: first, to ascertain whether or not foreign substances could be introduced into ovaries in such manner as to affect the ovules with a minimum of traumatic effects, so that the ovaries might reach maturity; and secondly, to ascertain whether or not such changes could be produced in an early stage of sexual specialization, before the development of the embryo-sac or after the union of the sexual elements in fertilization.

The first results were obtained with pure strains of *Oenothera biennis* and *Raimannia odorata* at the time mentioned, but the transfer of my activities from the New York Botanical Garden to the Desert Laboratory made it impossible to carry out cultures of the progeny or to repeat similar experiments upon this material. Meanwhile, Col. R. H. Firth, of the Royal Medical Corps of Great Britain, duplicated[1] my general results with *Raimannia* and other plants in 1908, although the fact that I had previously done this work was unknown to him.

New material was selected from the vicinity of the Desert Laboratory and the tests were begun anew in 1906. The difficulties to be overcome in such experiments are fully commensurate with the importance of the problem upon which they bear. It is a necessary preliminary that the plants chosen for the operations should be an elementary strain, a matter which may need two or three years for determination, if not already known. Next, not all ovaries will withstand the shock and injury inflicted in the operations. The chances of ultimate success will be greatest in many-seeded ovaries in which the number, however, does not extend much beyond that of ovules which may be affected by a single operation, giving some opportunity for differentiation of effects and not entailing large cultures. Lastly it is advantageous to deal with perennial species which come quickly to maturity. This gives

[1] Firth, R. H. Roy. Med. Corps, Jour. **16**: 497–514. 1911.

the operator opportunity to preserve the original material alive and to have it for comparison with succeeding generations.

The numerous cacti in the vicinity of the Desert Laboratory lead them to be selected for some tests, and the mechanical conditions for operation which they offer are unexcelled. As much as 1 cc. of solution may be introduced into the ovary of an opuntia without traumatic effects, but as all are under suspicion as to their genetic complexity, and as they germinate and develop slowly, the investigator must wait the greater part of a decade to obtain decisive results. Striking departures were obtained with *Echinocereus Fendleri,* a small cylindrical form native to southern Arizona, and the changed characters grouped in one derivative have not been obtained in nature or in cultures of the original. This derivative has been obtained a second time. The species, however, presents such a complexity of characters that definite conclusions are difficult.

Similar conditions were encountered in *Penstemon Wrightii,* about which an announcement was made in 1909. Some of these, however, furnished material from which the greatest sources of error might be eliminated.

The search for suitable subjects for experimentation was continued and the results with *Penstemon* led to a closer examination of other members of the *Scrophulariaceae.* Finally, an undescribed species of *Scrophularia* from the pine-forest area on the Santa Catalina Mountains in Arizona was brought into the environic series of the Laboratory of this Department in 1909. Rootstocks were taken to the Coastal Laboratory, and seeds were germinated at various localities. After having seen many hundreds of plants taken from various parts of its range and having followed them thoroughly two and three generations, it was found that the species is a simple one and not readily separable into elementary forms or strains. The only noticeable feature suggestive of complications was the fact that the broad-bladed nepionic leaf-forms are sometimes carried nearly to the summits of stems grown under certain conditions, giving the appearance of a robust race.

Another feature that received attention was the fact that branches formed in the closing part of the cycle of development of shoots bear leaves very much smaller than those arising from the median part of the main stem during the first part of the season. The flowers borne on these branches are also much paler than those on the more robust branches. Peloric flowers sometimes appear near the apices of the inflorescences in this as well as in other species of the genus. It is to be noted also that the divisions of the corolla are variously and irregularly incised on individuals at times during the season, but these are not heritable and do not appear in any regular manner.

This scrophularia appearing to offer some promise, several ovaries of a plant at Carmel were treated with solution of potassium iodide, one part in forty thousand, in July, 1911, and the ripened capsules were collected in September of that year. No record was made as to the time of day (see page 268) and nothing may therefore be said as to the possibilities of the action of the reagent on egg or pollen nuclei, singly, together, or after fertilization. No other species of *Scrophularia* grew near the cultures at that time.

The seeds were sown in suitable pans of screened soil, and in February three plantlets had survived. In May these were set in the open and their development followed. One formed a shoot fairly equivalent to the normal, finally producing flowers in which the anthocyans of the flowers were of a noticeably deep hue. The two remaining plantlets were characterized by a succulent aspect of the leaves, and by a lighter or yellow color of the leaves and stems. Inflorescences were matured late in 1912, and the flowers on one of the derivatives, as they may be called, were so completely lacking in color as to be a cream-white, this derivative being designated as *albida*, while the other showed some marginal color and a rusty tinge, and was designated as *rufida*.

Some disturbance of the relative velocities of development of the fibrovascular elements and mesophyll had taken place in both forms, so that the leaves were variously bowed and convexed and the two halves of the laminae were unequal and the whole blade was more oblique in outline. The elongation of the lamina had been checked and the ratio of width to length of the leaves was greater than in the parental stock. If correspondent leaves of *rufida* and the originals were laid side by side it could be seen that the basal veins on the side away from which the tips were curved were different in the two cases, the derivative showing two strong veins in the place in which one lateral with a thin branch occurred in the original (fig. 1). The water relations of derivatives and normal were not identical, and when young shoots or branches developed

Fig. 1. *O*, branching lateral vein in parental *Scrophularia; D*, branching vein replaced by two laterals in leaf of modified *Scrophularia*.

under similar conditions were detached, those of the derivatives flagged and wilted much more quickly than those of the normal.

The auxetic departures noted above also extended to the inflorescences, which in the original show a fairly regular basipetal development into thyrses. The derivatives, however, exhibited a rather irregular maturation of clumps of buds and the thyrses were very irregular, not reaching the spread of the parental forms. The fragility of the leaves does not seem to extend to the flowers, which opened very slowly, and in some cases the distended corolla persisted for a few days. The amount of color in the corolla was largely a matter of illumination, but under equivalent circumstances the derivatives always showed less than the parental form. As noted above the color persists to some degree in the derivatives along the margins of the uppermost lobes of the corolla, while that on the broad upper surface disappears. It is to be recalled that it is the color of this region which is variously disposed in other species of the genus.

The corolla lobes were irregularly incised in the flowers of the first and second seasons of the F_1, as they have been seen to be in the original, but in the second generation of both derivatives cultivated at the Desert Laboratory this effect persists as a regular wedge-shaped incision of the lower lip only, and is not seen in every individual of both derivatives, although the seeds were from plants which may have been pollinated by the parental form.

Seedlings from the original stock grown from seeds gathered on the Santa Catalina Mountains in Arizona were sowed early in 1910 at the Desert Laboratory and the plantlets preserved on April 15 furnished the data:

First pair of leaves smaller than in the derivative, being only 13–15.5mm. wide and 16–18mm. long, obscurely dentate with not more than two or three blunt teeth showing on each side. The petioles were 12–16mm. long. The third pair of leaves above the cotyledons, which probably were not quite mature, had petioles 20mm. long, and laminae 22–25x50–52mm. Marginal stalked glands were so numerous that 15–20 appeared in the field of the microscope at one time, and these structures were very numerous on the petioles. It is to be noted that differences in the last-named feature between this original and the derivatives disappear in the adult, or on the leaves appearing in the later stages.

Seeds from the original two derivatives matured at Carmel late in the summer of 1913 were sowed in the greenhouse at Tucson in November, 1913. But one plant of *albida,* the extremest departure, survived, while four of *rufida* were secured. These, of course, represented the F_2 of the departures. The measurements of *rufida* correspondent with those of the original are as follows: First three leaves deeply incised, five or six teeth on a side, abruptly pointed. Petioles 18–22mm. long, laminae 21–26mm. wide and 41–45mm. long. Mature leaves on sixth, seventh and eighth internodes, with petioles

36–45mm., and laminae 36–56x85–100mm. Marginal glands showing 6–10 in field, few on the petioles.

The single plantlet of *albida* bore leaves, the first pair of which were not deeply cut, the three or four teeth on each side being abruptly but sharply pointed, the petioles 15mm. long, and the laminae 24–26x35–38mm. The leaves from the sixth, seventh and eighth internodes had petioles 30–40mm., and laminae 45–51x90–100mm. Not more than four stalked glands might be seen in the field at any one time. These trichomes were very sparsely distributed over the under surface of the petioles only. The greater relative width of these leaves was correlated with a greater angle of divergence of the lateral veins from the midrib, a feature which, as will be shown later, was to be observed in adult plants.

The three plants representing the progeny of the treated individual were established in a row within a half meter of each other at Carmel in 1912. Irregular clusters of long thickened roots were formed, and these, as is customary with the species, bear buds and are a means of propagation of the plant. The three plants were taken up in November, 1913. While the main clumps could be identified, yet broken fragments of roots were preserved which could not be assigned to any one of the three, and although these were and are still preserved they are not taken into account here.

Albida was divided in May and June, 1914, and portions were sent to coöperators in New York, St. Louis and Chicago, but all failed to survive this unseasonable transplantation, so that at the present time this strain is represented by only two clumps, one of which is at the Desert Laboratory and the other at the Coastal Laboratory. The single plant of *albida* bloomed at Tucson early in the year, while the one at Carmel reached that stage too late to mature seeds.

Rufida was divided into three clumps and reset in the garden at the Coastal Laboratory in November, 1913. The shoots from these began to open flowers in July, 1914, which corresponded in all essential particulars with those of the previous seasons except that they were more highly regular. Two were enclosed in small glass cages for protection and to insure close pollination, a strong individual of the original being similarly enclosed for purposes of control. Conditions being favorable for a minute comparison of these plants with the parental type, colored illustrations of flowers and buds and diagram of structure were prepared. The inequality of the leaves was recorded by direct prints. The dimensional relations noted above were again seen. The readiness with which the leaves flag was noted and in these organs, as well as in the stems, it was seen that rigidity is maintained by turgidity rather than by stiffness of the mechanical tissues. The development of the bast-fibers is less marked in the derivative,

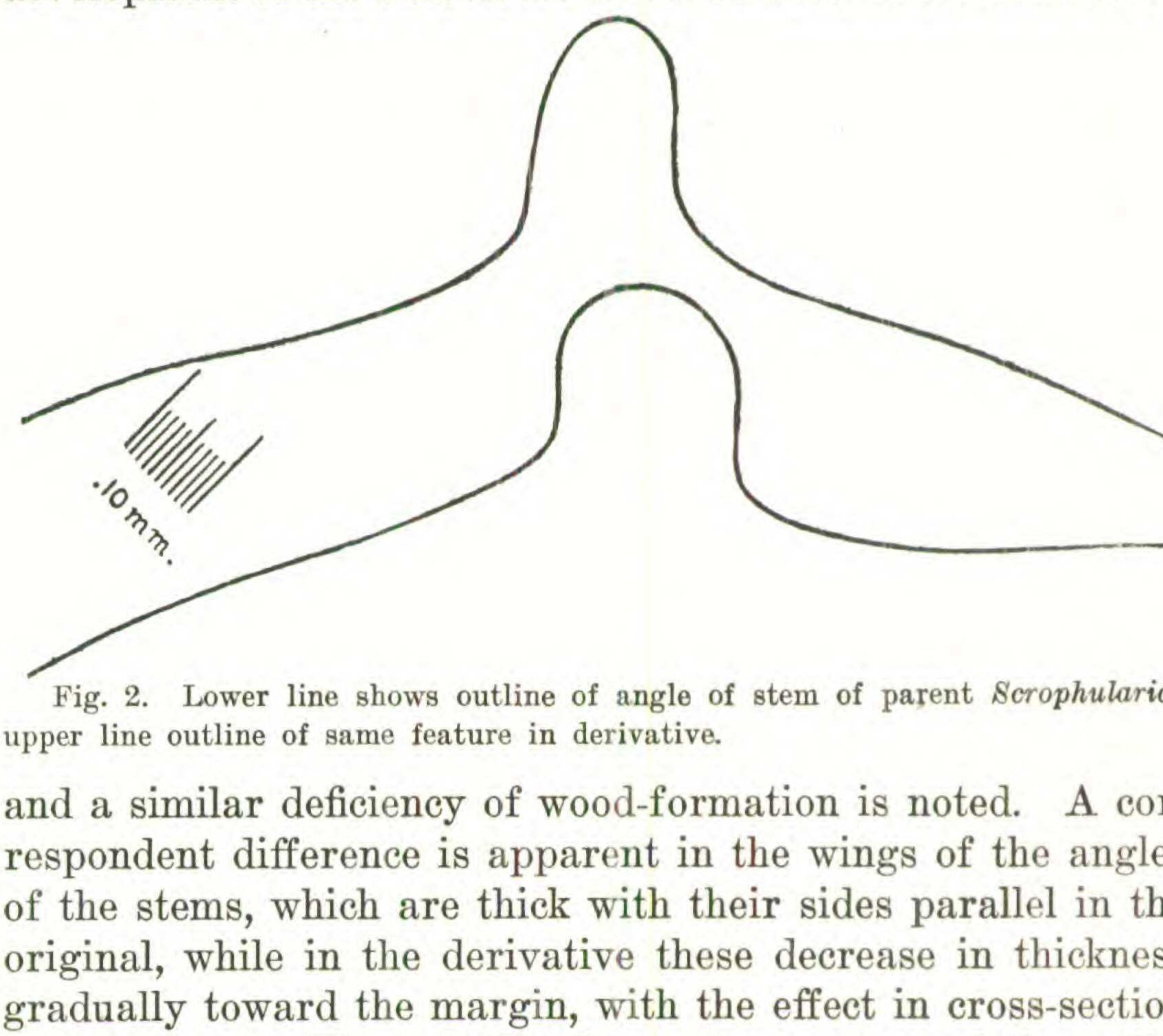

Fig. 2. Lower line shows outline of angle of stem of parent *Scrophularia;* upper line outline of same feature in derivative.

and a similar deficiency of wood-formation is noted. A correspondent difference is apparent in the wings of the angles of the stems, which are thick with their sides parallel in the original, while in the derivative these decrease in thickness gradually toward the margin, with the effect in cross-section seen in fig. 2. The actual value or importance of these differences is not a matter of moment in the present connection. The chief interest lies in the fact that recognizable effects have been produced by the introduction of foreign substances into ovaries and that the differences shown by the first generation, F_1, are borne by the second generation, F_2. The original observations with the plant in which this was demonstrated began in 1909, the treatments were made in 1911, and now first and second generations of the derivatives are alive, as well as the original stock.

Much irrelevant comment and inconclusive experimentation has followed the original announcement of the discovery of the methods used in this work. The necessity for a careful genetic analysis of the material for treatment has already been noted, and it may be well to call attention to some of the features of operation which might appear simple, yet are not easily carried out. No better way has yet been found for introducing solutions into the region of the embryo-sac than by injection into ovaries with an all-glass syringe fitted with gold needles (14 karat). The wounding of the ovary produces abortion in some species, and in almost all treatments some of the ovules are crushed. This, however, is a matter of no moment if some reached by the reagent survive and come to maturity. The extent and mode of diffusion of the reagent is in fact one of the most important features of the treatment, and the experimenter will do well to make control tests for the purpose of finding out whether or not there is some possibility of success.

A test of the ovaries of *Carnegiea* previously described showed that the liquid was taken up by the placental vessels and conducted to a point near the egg cell in a very short time if the reagent were introduced into the ovaries of flowers fully open and mature. Operations made at an earlier stage resulted in the accumulation of the reagent in the inner walls of the locule, in the integument of the ovule and especially at the micropylar orifice. The pollen tube would be subject to the action of the accumulated substance in the micropyle and integument in this case.[1]

It being my present intention to extend experimentation in the *Scrophulariaceae,* tests have been made with methylene blue in the ovaries of *Penstemon Torreyi,* the solution being one part of the dye to ten thousand of distilled water.

[1] MacDougal, D. T. Alterations in heredity induced by ovarial treatments. Bot. Gaz. **51**: 241–256. 1911.

Three hours later but little of the color could be found in sections of the ovary. Next, five ovaries of *Oenothera* 3.21

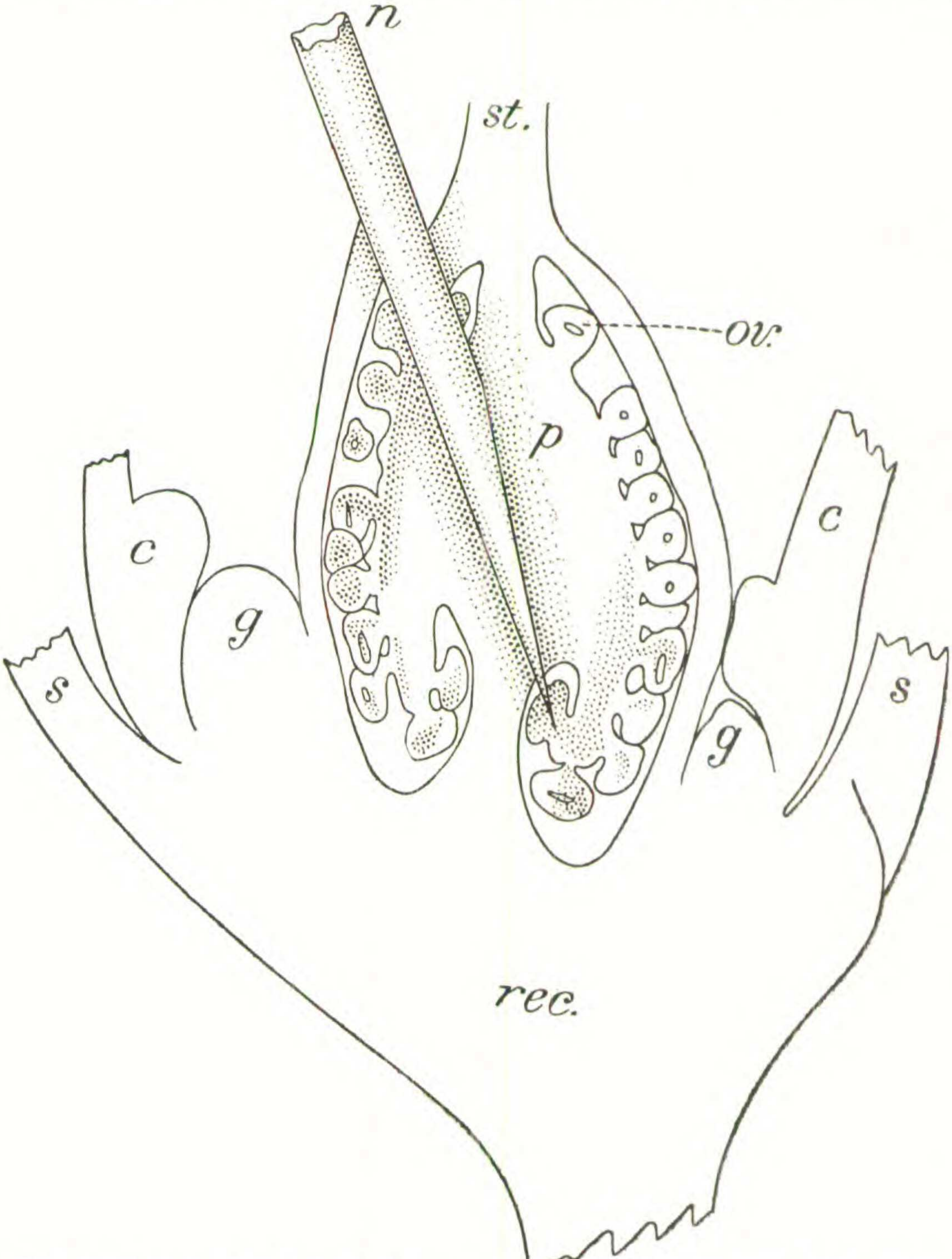

Fig. 3. Diagram of flower of *Scrophularia*, showing mechanical features of ovarial treatment: *s*, sepals; *c*, corolla; *g*, nectar gland; *p*, placenta; *st*, style; *ov*, ovule; *rec*, receptacle; *p*, tip of hollow needle thrust through the ovarial wall and penetrating the placenta. The stippling shows the diffusion of a solution of methylene blue introduced by the needle.—Drawn by F. E. Lloyd.

(a stable cruciate hybrid) were injected with a solution of one part in a thousand. Fifteen to forty ovules had been touched by the color in young flowers not yet open. A much larger number had been colored in the ovaries of mature flowers. This solution was introduced into ovaries of the scrophularia under examination (fig. 3). Young ovaries in this plant showed very few ovules affected, none in a few cases. Older ovaries in which fertilization had probably taken place showed as many as 15–20 colored ovules. Probably only a small proportion of the ovules affected would have survived and developed into viable seeds, so that many of the treated ovaries would have yielded nothing but normal seeds. This condition is to be taken into account by those who do not recognize the technical difficulties in the way of duplication of any particular treatment.

The recent results of Churchman and Russell[1] in securing stimulation of the growth of animal tissues with methylene blue suggest that this substance might produce some effects on the embryo-sacs of plants, and also the advantage of using a reagent the diffusion and penetration of which are visible and obvious.

It was desirable to use this dye in obtaining some knowledge of the probable action of other solutions in *Scrophularia,* so tests were made with this plant. A number of ovaries on a detached shoot in the laboratory were placed in a solution of one to a thousand at 9:30 a. m. Material was taken for examination at suitable intervals.

The placental walls and funicles were stained in part within a half hour. Two hours later the color had advanced well along the conducting tract in the funicular stalk. Five hours after treatment a notable amount of the dye had been carried clear to the embryo-sac, where it stained the nucellus and the antipodal region deeply. It is to be noted that the material was still alive and that this material if left attached to the plant would have developed some mature seeds in all probability (fig. 3).

[1] The effect of gentian violet on protozoa and on growing adult tissue. Soc. Exp. Biol. and Med., Proc. 2: 124. 1914.

Professor F. E. Lloyd, of McGill University, who kindly came to my aid in this matter, now made a brief study of the intra-vitam staining in the ovules of *Scrophularia* and found that the reagent accumulated throughout the embryo-sac inclusive of the egg cell, demonstrating the possibility of the direct action of introduced solutions on the entire egg apparatus as well as upon the endosperm. The micropylar orifice was closed and was not stained in the ordinary treatments and took up only a small amount of the dye when laid separately in a solution of it. Professor Lloyd also showed me preparations in which pollen tubes deeply stained had entered the micropyle and had elongated, reaching the egg.[1] These experiments made clear the immediate possibility of reagents reaching the egg apparatus through the funicle and of the staining of the pollen tube and nucleus in the cavity of the ovary before fertilization. It is also possible that the pollen tube might be affected by reagents which had accumulated in cells through which it penetrates to the egg nucleus (fig. 4).

These facts would make it probable that treatments before pollination has taken place would affect the embryo-sac and its inclusions only, while introductions of solutions at a later stage would be likely to affect the pollen tubes and nuclei. These generalizations are to be taken to be applicable to *Scrophularia,* and to species which present similar arrangements for reproduction. The egg in ovules in which the micropyle is open might be even more readily exposed to the action of a reagent, and if the ovule is porogamous the pollen tube would also inevitably be affected, and still many other combinations may be encountered which need not be enumerated at this time.

It is of course to be understood also that not all of the ovules in any pistil are in equivalent stages of development at any given moment, and this applies also to the penetration by the pollen tubes. Pollination of *Scrophularia* takes place in the morning, and substances introduced before mid-forenoon

[1] See Lloyd, F. E. The intra-vitam absorption of methylene blue in ovules of Scrophularia. Report of the department of botanical research for 1914. Carnegie Inst. Washington, Yearbook **13**: 77–81. 1914.

would be taken up and diffused through the tissues, especially through the funicle before the pollen tubes had reached the cavity of the ovary. Introductions timed to meet the elongat-

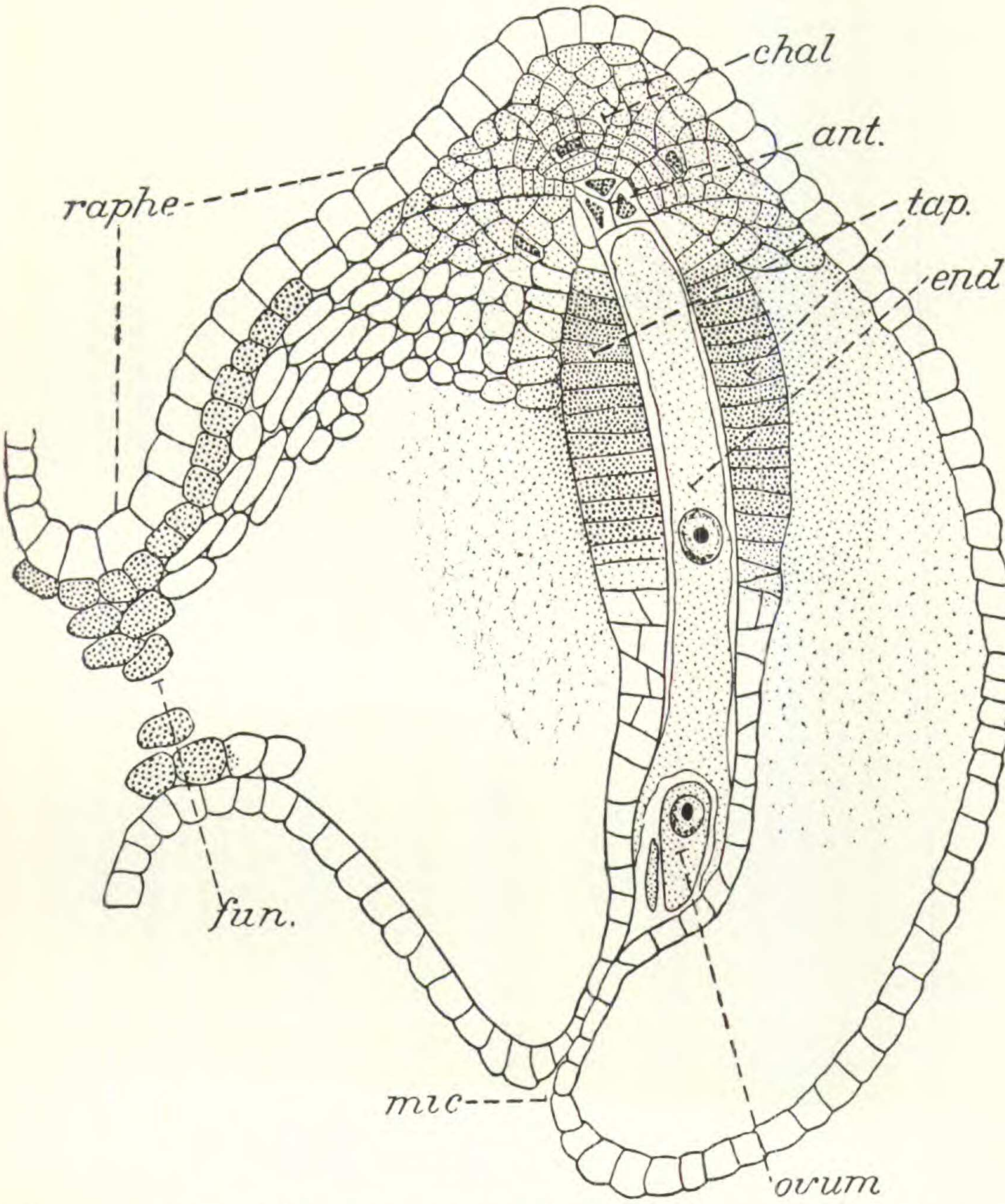

Fig. 4. Diagram of longitudinal section of ovule of *Scrophularia: fun*, funicle; *chal*, chalaza; *ant*, antipodal cells; *tap*, tapetum; *end*, endosperm; *mic*, micropyle. The shading shows the course of a solution of methylene blue diffusing through the funicle from the placenta (see fig. 3) and its selective fixation in the tapetum and nucellus. The solution finally reaches the ovum.

ing pollen tubes would of course be more liable to affect the pollen nuclei, and a number of lots of seeds matured in ovaries treated at various stages of development now await germination and test.

The differences between the two surviving derivatives of *Scrophularia* described in this paper may well be due to such differential action. It is to be seen that if egg or sperm were affected singly the resultant seed into which these elements might enter would be hybrid. Even if both were acted upon, it is by no means to be taken for granted that the effects in the two would be equivalent. The F_2 of *rufida* was identical in the cultures described, while the F_2 of *albida* presented some modifications, the status of which is not yet established, as both were open pollinated in the F_1. Very little information as to hybrids in *Scrophularia* is available. Goddijn and Goethart[1] report that *S. Neesii* Wirtg. × *S. vernalis* L. is a unified, stable, intermediate type and that the reciprocal is of a similar character.

The behavior of the original stock, and the facts of fertilization, yield nothing suggestive of parthenogenesis, and the derivatives may be taken to be produced by a typical fertilization. No cytological examination has yet been made for the purpose of ascertaining possible differences induced in the chromosomes.

This discussion may be fittingly brought to an end by a brief reconsideration of the salient ideas which have been touched upon. The point of view taken throughout all of the work which has been described is one in which the conception of a theoretical or idealized germ-plasm has been relegated to secondary position, and attention has been concentrated upon the concrete germ-plasm of the higher plants. This physical basis of heredity is seen to present two distinct phases. In one it takes the form of a meristem or embryonic tract of highly distended cells in which auxesis and division are both rapid and the elements which are separated from it pass by differentiations into the permanent tissues of the soma. Environic agencies affect only the development of the somatic cells which are being formed from the meristem, and the ex-

[1] Ein künstlich erzeugter Bastard Scrophularia Neesii Wirtg. × S. vernalis L. Van's Rijks Herb., Mededeel. 1913^{16}: 1–9. 1913.

perience of these cells are not reflected back to the embryonic tract, so far as available facts may be considered. Sexually specialized reproductive elements with a reduced number of chromosomes are developed from the embryonic tracts in a late stage of the ontogeny, and these elements present a metabolic balance different from that of the meristem stage, the colloids having a greater density, and some of the energy transformations having altered velocities.

The embryonic tract or meristem of a higher plant at any given moment includes an enormous number of primitive or initial cells and of separating elements in all stages of division, growth, and differentiation toward the specialized tissues which are derived from it. The tract as a whole could therefore not react in a unified manner to any climatic or environic agency which would impinge upon the plant. Such forces, as a matter of fact, visibly affect only the manner in which the differentiation of the resting tissues takes place. The rejuvenescence of such differentiated cells might carry the effects into the organ or individual produced by the regeneration, but no test has yet been made of this matter, or of the transmission of such supposititious characters to a second sexually produced generation; neither has the proposal, that repeated or long continued exposure of the germ-plasm to any environic stimulus may result in the fixation of effects, been tested out. The continuation of introduced species in the mountain, desert, and coastal plantations of the Department of Botanical Research for the term of years during which any one person might conduct such experiments, may not be taken as an adequate test of this phase of the matter, although these cultures are carried on for the express purpose of determining what permanent changes may be induced by the tension of unusual environic complexes. So far these have been confined to alterations in sexual and asexual reproductive procedure, and to alterations in structure and aspect of the shoot, while no tests have been made upon the fixity of the changes.

Aberrant behavior of the chromosomes in certain determinative or initial cells may possibly be responsible for bud-mutations or bud-variations, and theoretically it is conceivable that special stimuli might be applied to such cells in a manner that might bring about similar results. Practically, however, it would be enormously difficult to localize initial cells with sufficient certainty so as to give any slight chance of success.

The second stage of germ-plasm in which it is in the form of sexually specialized elements offers far more promising conditions for experimental modification of the genetic content of the species which it represents. Solutions may be introduced into the ovaries in such manner as to affect the egg bearing the entire group of qualities of the species, and furthermore the direct action of such reagents may be ascertained to some extent.

The present-day aspect of the mechanism of heredity is one which increases momentarily in complexity. The greater part of the researches in genetics during the last fifteen years has been devoted to the interaction of factors, determiners, inhibitors, or qualities in the organism. If these conceptions may be taken to be the expression of the reactions of either chemical groupings or to rest upon a physico-chemical foundation of any kind, the reagents which have been used have not been of a selective character, but would affect practically the entire colloidal mass of the protoplast in some manner and to varying extent, neutralizing or coagulating proteins, and their general tendency would be to inhibit or check energy transformations. In the case of the iodine treatments the free ions from potassium iodide or the iodic acid formed would cause a neutralizing effect, as it does not seem from the results of Czapski and Adler[1] that this element would form any compound with the proteins.

The experimenter is dealing with an actual physico-chemical complex of highly unstable compounds in which many types of energy transformation are occurring. Introduced substances may slow down or inhibit some of these, and accelerate others or start new reactions. The morphological possibilities in any given strain of plants are somewhat limited, however, and in this sense the direction of the departures is al-

[1] Beiträge zum Chemismus der Jodwirkung. Biochem. Zeitschr. **65**: 117. 1914.

ready determined. This limitation of the possibilities of morphogenesis is the chief one in any expectancy of duplication of results in successive treatments, outwardly mechanically identical.

The variables in any experimental setting are many, and the briefest consideration of the physical effects consequent upon the introduction of a foreign solution to the vicinity of the embryo-sac, reveals at once the lack of probability of exact repetitions in a mechanism so complex. The conditions are much different from those which would be presented if free floating eggs or sperms were immersed in a solution. If we are able to induce other changes in *Scrophularia* besides those shown, they will be quite as important in demonstration of the fact that germ-plasm had been modified as if they were exact repetitions of previous inductions. If previous results were exactly recalled there might be some suggestion of premutation.

It is evident that the experimenter who wishes to proceed with the greatest precision and least loss of effort will first test the genetic strictness of his living material, ascertain the rate and manner and diffusion of solutions in the ovary and ovules, the time of pollination and the rate of development of the tube in reaching the egg. Next, the structure and number of ovaries and the traumatic reactions of the entire pistil are to be taken into account. Having also traced out the simpler features of pollination and fertilization, the operator should test the effects of various reagents which may neutralize proteins, including enzymes, or act as excitators or catalyzers. Without enlarging too much upon the difficulties to be encountered in the experiments described in this paper, they may be illustrated by the fact that over fifty operations upon *Scrophularia* in July, August and September, 1914, at Carmel, California, were total failures, as the ovaries perished before reaching maturity.

Finally, many present interests in phylogeny and genetics will be concerned with the nature of the evolutionary movement which is simulated by the alterations which have been induced experimentally by the method described. Some of these would unquestionably be designated as of a retrogressive character, such, for example, as the defection of a part of the color pattern of the corolla; others, such as the accentuated incision of the leaves and corollas and the development of the venation, as progressive alterations; while still others may not with any substantial reason be assigned to either class. With reference to taxonomic criteria, it may be said that the divergent individuals are distinguishable at sight from the parental stock, but the real test of the characters presented is not their degree or kind of departure, but their stability and permanence indicative of actual modifications of the germplasm.